THE USE OF PHYLOGENETIC PERSPECTIVE IN COMPARATIVE PLANT PHYSIOLOGY AND DEVELOPMENTAL BIOLOGY[1]

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ABSTRACT

The use of phylogenetic reconstruction for the testing of comparative hypotheses is a recent development in the fields of plant physiology and developmental biology. In this review, several uses of phylogenetic information are discussed, including (1) the use of phylogenetic trees for choosing experimental systems, (2) justifying the statistical independence of compared taxonomic groups, (3) identifying evolutionary direction using outgroup analysis, and (4) studying the evolutionary tempo of physiological and developmental change. Difficulties are also discussed, especially with respect to (1) mapping continuous, physiological traits onto the discrete, binary structure of phylogenetic trees, (2) constructing trees from traits that are independent of the physiological and developmental characters being mapped, and (3) respecting the statistical nature of phylogenetic trees. The bulk of the review is devoted to several examples from the literature that illustrate the power of a phylogenetic perspective in comparative studies. Reviewed examples include (1) elucidation of a novel pyrophosphate-dependent phosphofructokinase in the glycolytic pathway of phosphate-stressed plants in the Brassicaceae, (2) evolutionary pattern in the origins of polyploid endosperm in angiosperms, (3) adaptive radiation of Hawaiian Island plants into dry habitats, and (4) evolutionary pattern in the origins of C_4 photosynthesis. The fields of systematics, comparative physiology, and developmental biology will continue to merge as their common reliance on phylogenetic perspective increases.

The purpose of this review is to describe aspects of the past, present, and future relationship between two historically disparate disciplines—comparative plant systematics and comparative plant physiology and development. Researchers in plant systematics focus on the pattern of evolutionary diversification. Researchers in plant physiology and plant developmental biology focus on the products of evolutionary diversification—functional mechanisms, structural attributes, and their relationships to genetic and environmental controls. The nexus among these disciplines occurs through a need to understand the role of evolutionary constraint in dictating patterns of adaptation, the timing and rate of phenotypic evolution, and the evolutionary interactions among phenotypic traits.

Historically, one can recognize several connections between the fields of comparative systematics and comparative functional biology. As examples, consider the numerous studies that originated in Sweden with Turreson (1922) and in the United States with Clausen et al. (1940, 1948). The focus of these studies was firmly embedded in the evolutionary process: to uncover the cohesive and divergent forces that maintain the structure of taxonomic units while at the same time allowing for diversification and speciation. A principal component of these early studies included comparisons among plants from different geographic locations that were grown in common environments. The goal pursued in these "common-garden" studies was to partition the influence of genetic and environmental influences on character expression. During the birth of modern comparative physiology, and to a lesser extent developmental biology, scientists discovered that the same common-garden approach could be used to standardize the influence of environment, while observing inherited differences in the adaptive responses of plants (e.g., Mooney & Billings, 1961; Björkman & Holmgren, 1963). Fur-

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thermore, by choosing congeneric and confamilial groups for their comparisons, these mechanistic biologists were using phylogenetic perspective as they observed the products of common descent, identified examples of convergent evolution, and isolated patterns of adaptation. Thus, at its inception, modern comparative plant physiology had definitive links with comparative plant systematics. Unlike researchers in comparative systematics, however, the focus of comparative physiologists was not the evolutionary process in relation to natural taxonomic units, but rather the nature of mechanistic adaptations and their relationship to environmental extremes.

Since these pioneering studies, researchers in physiology and developmental biology have strayed from their phylogenetic, comparative origins, embracing instead the implicit assumption that functional responses to the environment are the products of natural selection. When it comes to interpreting evolutionary patterns in functional traits, formal hypothesis testing has often given way to intuition and adherence to an adaptational doctrine. This tendency has been criticized in past essays (e.g., Gould & Lewontin, 1979). The potential contributions of phylogenetic reconstruction and the comparative method to formal hypothesis testing in physiological and developmental biology have only recently been revisited (e.g., Felsenstein, 1985; Huey, 1987; Harvey & Pagel, 1991; Garland & Carter, 1994; Lauder et al., 1995). There is little doubt that as more biologists become aware of the useful deductions that can be made using phylogenetic data, a remarriage will be witnessed between the fields of comparative systematics and comparative physiology and development.

ON THE DIFFERENT NATURES OF COMPARATIVE SYSTEMATICS AND COMPARATIVE PHYSIOLOGY/DEVELOPMENT

In comparative systematics, trait variation is used to study natural relationships among different groups of organisms. In comparative physiology and development, natural relationships among organisms are used to study the nature and functional significance of traits. Thus, although researchers in both fields draw upon the same puzzle pieces (variation in organismic traits), those in one discipline use the pieces to assemble the puzzle, while those in the other discipline use the completed puzzle to study the pieces.

In focusing on adaptation, comparative physiologists ask the question: does physiological variation among organisms show a pattern that is consistent with the process of natural selection (Feder, 1987)? Pattern is typically measured as the functional attributes of organisms native to different environments. If the pattern correlates with enhancement of growth and persistence across an environmental gradient, then the pattern is ascribed to natural selection and is taken as adaptive. Recent studies, however, have revealed phylogenetic history to be as likely a constraint on pattern variation as is natural selection (Huey, 1987; Garland & Carter, 1994; Lauder et al., 1995). Disentanglement of phylogeny and selection is only accomplished through reliance on comparative systematics and the existence of accurate phylogenetic reconstruction. The integration of comparative systematics with comparative physiology and development is an exercise in mapping functional traits onto established phylogenetic trees. Several methods have been developed to accommodate such mapping and the accompanying statistical analysis (Felsenstein, 1985; Harvey & Pagel, 1991; Brooks & McLennan, 1991).

There are some potential pitfalls that must be recognized before one can successfully engage in the activity of phylogenetic mapping. For example, many comparative physiologists and developmental biologists may not recognize the tentative nature of phylogenetic trees. At first appearance, such trees reflect firm, definitive relationships, especially from the perspective of mechanistic biologists who are accustomed to observing the discrete conclusions of manipulative experiments. It is important, however, to recognize the statistical nature of phylogenetic trees. A phylogenetic tree is essentially the solution of greatest parsimony, given knowledge about a particular set of measured, shared traits among an assumed set of related taxa. In many cases a tree constructed from assumptions of maximum parsimony will poorly reproduce the alleged phylogeny (Fiala & Sokol, 1985; Rohlf et al., 1990; Lamboy, 1994). Phylogenetic trees have conclusive confidence limits that should be honored when framing conclusions about evolutionary patterns.

As a second example of issues dealing with phylogenetic mapping, comparative physiologists and developmental biologists must recognize disparities in the nature of traits that are frequently studied. Comparative systematics is based on the use of discrete, binary traits, the raw material of cladistic construction. Comparative mapping of physiological and developmental traits also requires that they have clearly delineated limits. There are examples of such traits (e.g., the presence or absence of the C_4 photosynthetic pathway), though most physiological traits, and to a lesser extent developmental
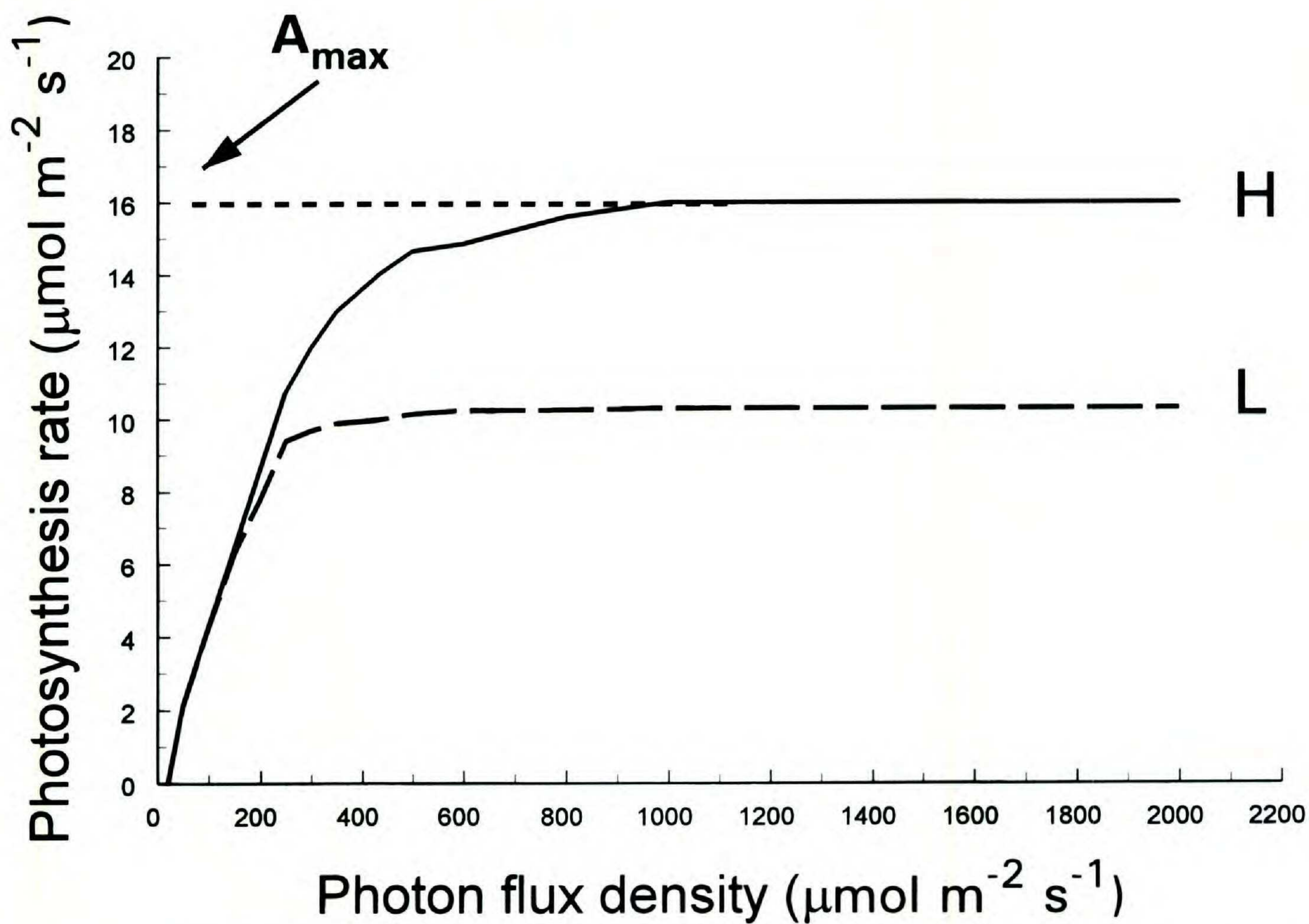
Figure 1. Hypothetical responses of photosynthesis rate to incident photon flux density in a high-light grown leaf (H) and a low-light grown leaf (L). The results demonstrate variability in photosynthesis rate as a function of the immediate (or instantaneous) light environment and the past (or growth) light environment. One commonly used index of photosynthesis rate in studies of genotypic differences is the maximum photosynthesis rate (A_{max}).

traits, are continuous and show plasticity in their responses to the environment. This leads to difficulties in the process of mapping. As an example consider the case for photosynthesis rate, one of the most commonly studied characters in comparative physiology. Within a plant, maximum photosynthesis rates can vary considerably (by up to a factor of 10) among leaves that develop in different parts of a plant's canopy (e.g., Fig. 1). By studying the cause of such variation comparative physiologists can gain insight into interactions between the photosynthetic process and a plant's environment, in this case the light environment. Yet this same variation creates difficulties in assigning discrete character values for phylogenetic mapping.

In some way, the influence of environment on character expression must be standardized. Phylogenetic reconstruction is only meaningful if the mapped traits reflect genotypic comparisons without environmental influence on character variability. One way this is commonly accomplished in studies of comparative physiology is to compare maximum observed values for some process (e.g., A_{max} in Fig. 1). It is reasoned that if the expression of a trait is measured at its maximum for all compared taxa, then variability in the measurements reflect genotypic differences. In practice, difficulties arise in determining the species- and trait-specific conditions that foster maximum expression of a trait. For some traits (e.g., resource acquisition rates) maximum expression may come with environmental conditions that maximize growth, whereas with other traits (e.g., mechanisms of stress tolerance) it may come under environmental conditions that minimize growth.

One final issue to deal with in terms of phylogenetic mapping is the need to construct phylogenetic trees from traits that are not mechanistically linked to those being mapped. This concern is minimized when trees are constructed from molecular markers (though one can imagine gene sequences in which molecular variability is reflected in enzymatic variability). Trees based on morphological traits carry greater risk in this respect. Lack of independence between traits used in tree construction and those mapped onto the tree would decrease the likelihood of reproducing the true phylogeny for the mapped trait.

On Specific Issues That Can Be Addressed Using a Phylogenetic Perspective

PHYLOGENY AND THE CHOICE OF EXPERIMENTAL TAXA

Phylogenies can be used to provide direction in the choice of experimental systems—allowing for the choice of taxa that are closely related or more distantly related. This allows investigators to maximize the possibility that the traits being compared are the products of common descent in the case of closely related groups, or convergent evolution in the case of distantly related groups. Through the a priori choice of groups with contrasting phylogenetic patterns, investigators can study the relative roles of phylogenetic inertia versus selection in influencing the evolution of functional traits.

Traditional choices of experimental systems in comparative functional biology have been driven by considerations of environment (Huey, 1987; Lauder et al., 1995). With respect to plants, the paradigm of choice has stated that adaptive responses to environment are best observed in extreme habitats. Thus, adaptive responses to high temperature are best studied in hot, desert habitats, whereas adaptive responses to cold temperatures are best studied in cold, tundra environments. This approach contains a compelling dose of intuition and logic. However, it also perpetuates some dangerous and shortsighted assumptions. One principal assumption is that any intuitively beneficial aspect of a functional attribute is the product of selection in response to the most common, and potentially stressful, environmental extreme. A corollary to this assumption is that, with respect to influences on phenotype, selection in a plant's current environment has overshadowed historical events in the acquisition of traits from past ancestors. These assumptions feed the adaptationist program discussed above. This current environment-oriented approach has little provision for phylogenetic constraint or inertia. This is where a phylogenetic perspective can contribute to adaptive analysis. By choosing groups based on criteria of both environment and the availability of phylogenetic reconstructions, one can conduct truly synthetic analyses of (1) the relative roles of selection versus phylogenetic constraint, and (2) the relationship of traits to the current versus past environment.

PHYLOGENY AS A GUIDE TO STATISTICAL ANALYSIS

Phylogenetic relationships can be used to justify statistical patterns among experimental groups being compared. It is an unattainable goal for comparative biologists to obtain groups that are com-

pletely independent in terms of past history. There is always going to be some degree of common, hierarchical descent in a system characterized by a monophyletic cladogram. Phylogenetic connections interfere with statistical assumptions of independence (Clutton-Brock & Harvey, 1984; Felsenstein, 1985; Martins & Garland, 1991). Basically, this is a "degrees of freedom" issue that becomes especially relevant in attempts to construct correlations among co-occurring traits or relate the expression of a trait with environmental variability. By ignoring phylogenetic relatedness, investigators tend to inflate the potential for Type I errors (wrongful rejection of the null hypothesis), reduce the power of their statistical conclusions, and increase inaccuracies in estimating correlation coefficients (Martins & Garland, 1991). The only groups that appear to be immune from such phylogenetic influences are those characterized by early diversification followed by long periods of phylogenetic stasis (so-called "star" phylogenies in Felsenstein, 1985) (see Martins & Garland, 1991).

Several methods have been proposed to deal with the problems of phylogenetic interdependence. The simplest method, though not the most accurate, is to center the analysis on higher taxonomic units, which will presumably possess weaker phylogenetic interdependence in terms of the traits they exhibit (Crook, 1965; Clutton-Brock & Harvey, 1984). This method does not completely deal with the issue of phylogenetic connections, however, since even the higher levels will be interdependent to some degree. An approach that deals more directly with phylogenetic connections was first proposed by Felsenstein (1985) and modified in various ways by others (e.g., Huey & Bennet, 1987; Grafen, 1989). In essence, this approach takes advantage of expected variance of character change to compute standardized, independent contrast values from the measured comparative data. Such contrast values can then be subjected to standard statistical tests of significance. Even with this approach, however, uncertainties exist due to limited knowledge about phylogenetic branch lengths and their relationship to the rate and pattern of evolution—specifically, whether trait diversification has occurred in a gradual or punctuated pattern (Martins & Garland, 1991).

PHYLOGENY AND THE DETERMINATION OF EVOLUTIONARY PATTERN

Phylogenetic trees represent maps from which one can polarize patterns of adaptive diversification. Polarization involves determination of the se-
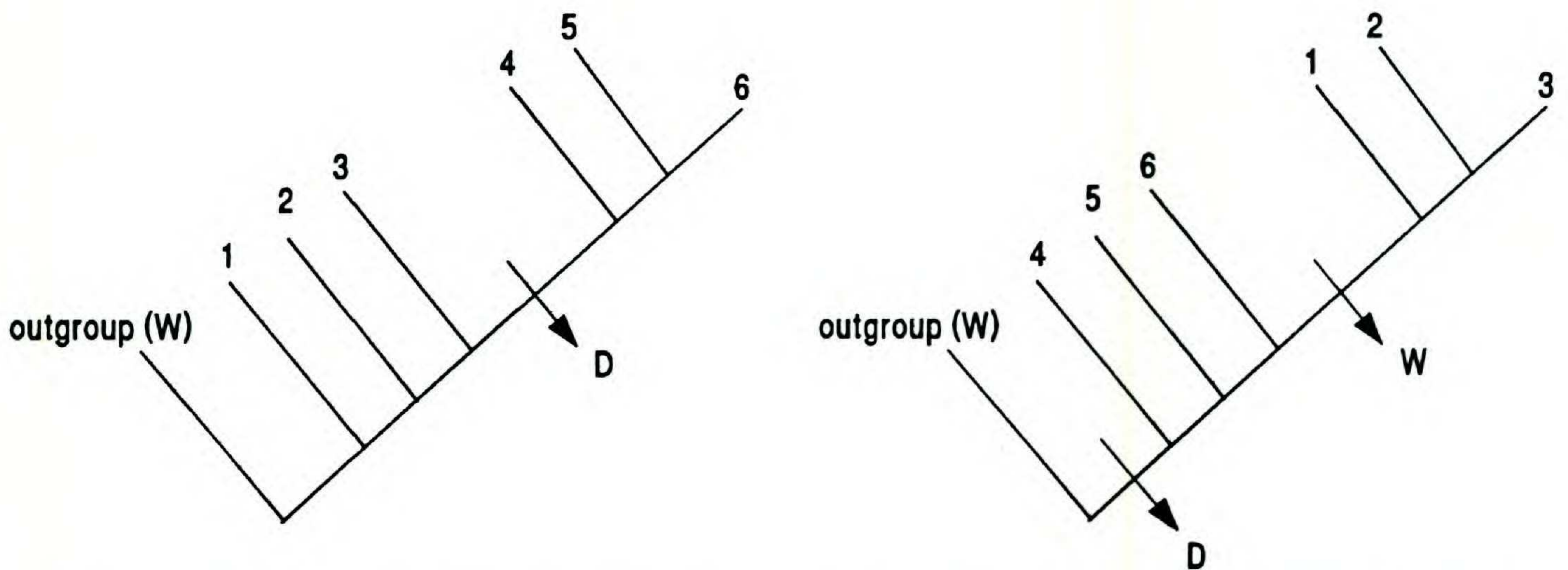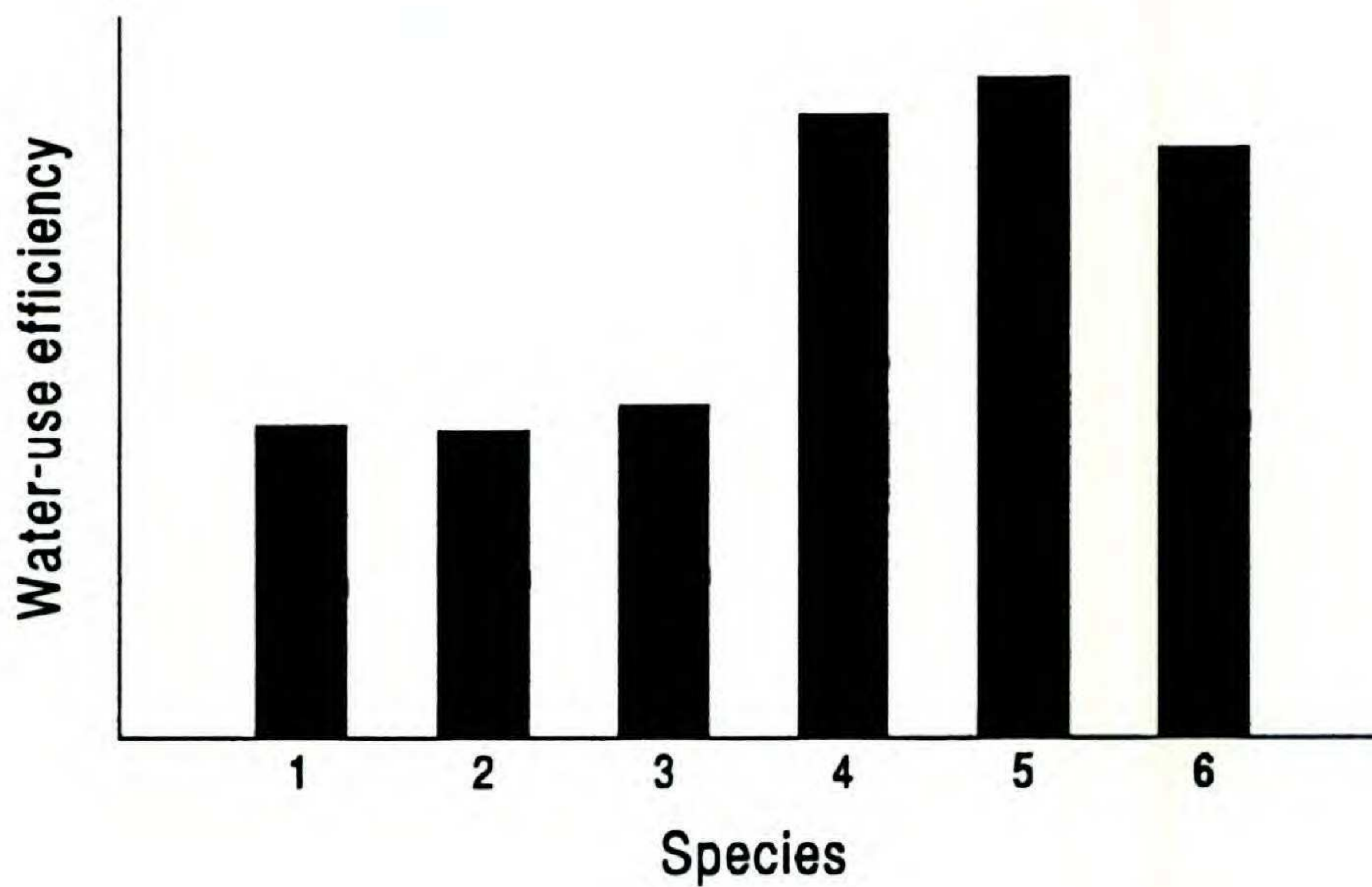
Figure 2.   Hypothetical water-use efficiencies measured for six different plant species (upper panel). The lower two trees demonstrate how evolutionary patterns in this physiological character can be polarized with respect to ancestral and derived traits using outgroup comparison. In both cases the outgroup is assumed to occur in wet habitats (W). Those species exhibiting lower water-use efficiencies are assumed to also occur in wet habitats, whereas those that exhibit higher water-use efficiencies occur in dry habitats (D). In the tree on the left, a single transition is found to occur as species radiated into dry habitats and evolved higher water-use efficiencies. In the tree on the right, a transition has occurred early during diversification of the group, followed by a later reversal.

quential origins of traits relative to patterns of taxonomic diversification. The origins of traits are polarized through comparison with ancestral outgroups. Without outgroup polarization, it is impossible to determine whether a trait represents the ancestral or derived state. This use of phylogeny is illustrated in Figure 2 for a hypothetical study of photosynthetic water-use efficiency. Without phylogenetic analysis, it is not possible to determine whether high or low water-use efficiency represents the ancestral state in this group of species. Two alternative phylogenies are presented, each of which leads to a different conclusion concerning the evolutionary pattern in this trait.

In addition to polarizing single traits, outgroup analysis and phylogenetic mapping also provide a means for studying the evolutionary interactions between, or among, multiple traits. By simultaneously mapping two or more traits, one can gain insight into how the evolutionary appearance of one trait may have influenced the appearance of a different trait. Alternatively, the appearance of traits can be mapped simultaneously with major ecological shifts (e.g., shifts in habitat type, phenology, or community structure). With respect to plants, this approach may be particularly useful in deducing the relationships between functional and life-history traits, as well as between functional and structural traits.

The use of phylogenetic trees for the study of evolutionary patterns represents the most fundamental of all symbiotic connections between the disciplines of comparative systematics and comparative functional biology. This is the connection

that is destined to draw the most attention from comparative physiologists and developmental biologists. As is evident in the following sections, this approach has great power to provide new insight into the processes and patterns of functional adaptation.

PHYLOGENETIC RECONSTRUCTION AND THE
DETERMINATION OF EVOLUTIONARY TEMPO

With appropriate calibration, phylogenies can be used to assess rates of evolution. Rates of evolution can provide insight into patterns of adaptive radiation and their underlying processes such as natural selection and genetic drift. Temporal calibration of phylogenies can be accomplished through paleontological dating, the use of molecular clocks (e.g., dePeer et al., 1993), or the application of statistically based trajectory models to the variance among measured characters (e.g., Lande, 1985; Lynch, 1990; Martins, 1994). The use of dated phylogenies to derive temporal patterns of evolution in functional traits has not been great. There are several complexities that still need resolution before such use is likely to increase. For example, molecular clocks are typically based on neutral traits. However, it is likely that most functional traits have, at some time in their past, been the subject of selection—a process that may have accelerated evolutionary change relative to neutral markers. Additionally, there are uncertainties in the clocks themselves and their application to phylogenetic reconstruction. These uncertainties include heterogeneity in the rate of nucleotide base substitution depending on the DNA environment (Saccone et al., 1989), errors due to interactions between the choice of nucleotide sequence and the method of tree reconstruction (Zharkikh & Li, 1993), and the influence of certain functional traits (e.g., generation time and metabolic rate) on nucleotide substitution rates (Martin & Palumbi, 1993). If such complexities can be resolved, evolutionary clocks could become valuable tools as comparative biologists address the issue of future environmental change and its influence on evolutionary patterns in the Earth's biota.

ON THE USE OF SYSTEMATIC INFORMATION
SYSTEMS FOR THE DISCOVERY AND INVENTORY OF
UNIQUE BIOCHEMICAL AND PHYSIOLOGICAL
PROCESSES

Phylogenetic trees provide maps of historical linkages among groups of organisms. As with any map, phylogenetic trees can provide direction and orientation for searches of unique functional pro-

cesses. It is not uncommon to find investigators screening an entire genus to determine the extent of distribution in some functional trait or identify possible comparative systems in which the trait differs. Such an approach has been taken, for example, in the identification of *Chlorella* species possessing the ability to utilize bicarbonate as an inorganic carbon source (Miyachi et al., 1985) and the assessment of phytochelatin (heavy-metal binding peptides) distribution within various groups of plants (Gekeler et al., 1989). Recognition of phylogenetic pattern in the distribution of phytochemicals has existed for many years. In fact, phytochemical distribution represents the foundation for chemosystematics, a central discipline within the broader field of comparative systematics. In many instances the chemicals of systematic interest also have roles in plant adaptation. Terpenes and alkaloids, for example, exhibit strong phylogenetic affinities and serve an adaptive role in deterring herbivory (Banthorpe & Charlwood, 1980; Lerdau et al., 1994). Quaternary ammonium and tertiary sulfonium compounds are distributed along conservative phylogenetic lines and have important roles in plant responses to salinity and water stress (Rhodes & Hanson, 1993). Numerous other examples of phytochemicals that have dual importance to comparative systematics and comparative physiology can be described (e.g., flavonoids, cyanogenic glycosides). In all these examples the systematic approach has played an obvious role in expanding the list of species that possess the chemical of interest and in establishing correlations between taxonomic and ecologic distributions.

One recent case in which phylogenetic knowledge has had an obvious influence on trait discovery concerns the inducible pyrophosphate-dependent phosphofructokinase that was recently reported in *Brassica nigra* (L.) W. D. J. Koch (Theodorou & Plaxton, 1993, 1994). Theodorou and Plaxton (1994) suggested that this enzyme confers an adaptive advantage under phosphate stress by allowing glycolytic processing of fructose 6-phosphate in the face of reduced ATP availability. Following a protocol typical of comparative physiologists, Plaxton's group recently screened other plant species to assess overall distribution of the inducible enzyme (W. Plaxton, Queen's University, pers. comm.). Their search revealed its presence at relatively high constitutive levels in tobacco and tomato cells, but the absence of phosphate-stress inducibility. A similar pattern of constitutive presence, without inducibility, was found in other *Brassica* species thought to be closely allied with *B. nigra* (e.g., *B. oleracea* L. and *B. rapa* L.). Thus,

it appeared that the induction mechanism was isolated to *B. nigra*, being absent even from congeners. At this point Plaxton's group initiated discussions with Suzanne Warwick, a systematist who had recently constructed a phylogenetic tree of *Brassica* and related taxa through the use of cpDNA markers (Warwick & Black, 1993). From the molecular phylogeny it was clear that *B. nigra* is more closely allied with three members of the genus *Synapis*, than *Brassica* (Fig. 3). Plaxton's group has recently surveyed the two species of *Synapis*, *S. alba* L. and *S. arvensis* L., which appear to share close affinities with the re-aligned *B. nigra*. Both *Synapis* species exhibit the inducible pyrophosphate-dependent phosphofructokinase.

This example provides three important conclusions concerning the use of phylogenetic trees in studies of comparative physiology and developmental biology. First, it is clear that phylogenetic trees can be useful in directing the search for novel biochemical, physiological, and developmental processes. Second, this example illustrates the potential two-way exchange of information in collaborations between systematists and functional biologists. In this case the distribution of the pyrophosphate-dependent phosphofructokinase provides independent support for the taxonomic realignment of *B. nigra* as suggested by the molecular phylogeny. Finally, it should be clear that the effective use of systematic information systems by comparative physiologists and developmental biologists requires that taxonomy reflect phylogeny. The fact that *B. nigra* was traditionally classified with *Brassica*, rather than with *Synapis*, caused this biochemical search to stray from the correct phylogenetic path.

One area in which a phylogenetic approach is currently missing, but potentially beneficial, is the study of herbicide resistance in weedy, agricultural pests. In the past 25 years it has become obvious that the continued use of herbicides on agricultural fields has resulted in the evolution of several herbicide-resistant weed species (Warwick, 1991; Holt et al., 1993). Apparently, there is considerable interspecific and interpopulation variability in the likelihood of evolving herbicide resistance. It is unknown to what extent such variability is due to differences in selection regime, population genetic structure, or phylogenetic constraint. A systematic approach would be of obvious benefit in partitioning the influences of selection versus phylogeny, though to date no such approaches have been attempted. An example of the potential use of comparative systematics to the question of herbicide resistance can be seen in the case of triazine resistance in *Amaranthus*. Within North America numerous cases of triazine resistance have been reported in *A. powellii* S. Watson and *A. hybridus* L., the two less widespread species of North American weedy amaranths (Hill, 1982). Triazine resistance has only been reported in one population of the more widespread species, *A. retroflexus* L. Although the data are incomplete, there is reason to hypothesize that the two minor species will usurp the ecological dominance of the widespread species in the face of continued heavy herbicide use (see Gressell & Segal, 1982). This issue is complicated by the fact that European populations of *A. retroflexus* appear more likely to develop triazine resistance compared to North American populations, a situation common to weedy species that find themselves outside their native ranges (Gressell & Segal, 1982). This problem begs for the inclusion of a phylogenetic perspective. The question of whether one species is more or less constrained by its phylogeny to evolve herbicide resistance would appear to be fundamental to understanding and predicting future patterns in biological responses to herbicide applications.

ON THE USE OF PHYLOGENETIC ANALYSIS TO DISCERN EVOLUTIONARY PATTERNS IN PLANT DEVELOPMENT AND FUNCTION

Phylogenetic trees provide comparative biologists with an important tool to uncover and polarize evolutionary pattern in functional traits. Through an examination of pattern comes insight into the evolutionary constraints that have influenced, and will continue to influence, functional responses to the environment. In the following paragraphs examples are provided to illustrate the use of phylogenetic trees to study evolutionary patterns in plant physiology and development.

EPHEDRA AND DOUBLE FERTILIZATION

For many years it was thought that a defining trait of angiosperms was the process of double fertilization during reproduction. Friedman (1990) has recently challenged this dogma through definitive observations of double fertilization in *Ephedra*, a non-flowering seed plant. Phylogenetic trees constructed from numerous traits, including molecular markers, have placed *Ephedra* as a basal member of the Gnetales, the group of extant non-flowering seed plants most closely allied to the angiosperms (Doyle & Donohue, 1986). Using these phylogenetic relationships as a guide, Friedman (1992) was able to demonstrate that the likely homolog to angiosperm endosperm is the supernumerary-embryo product of the second fertilization in *Ephedra* (Fig.
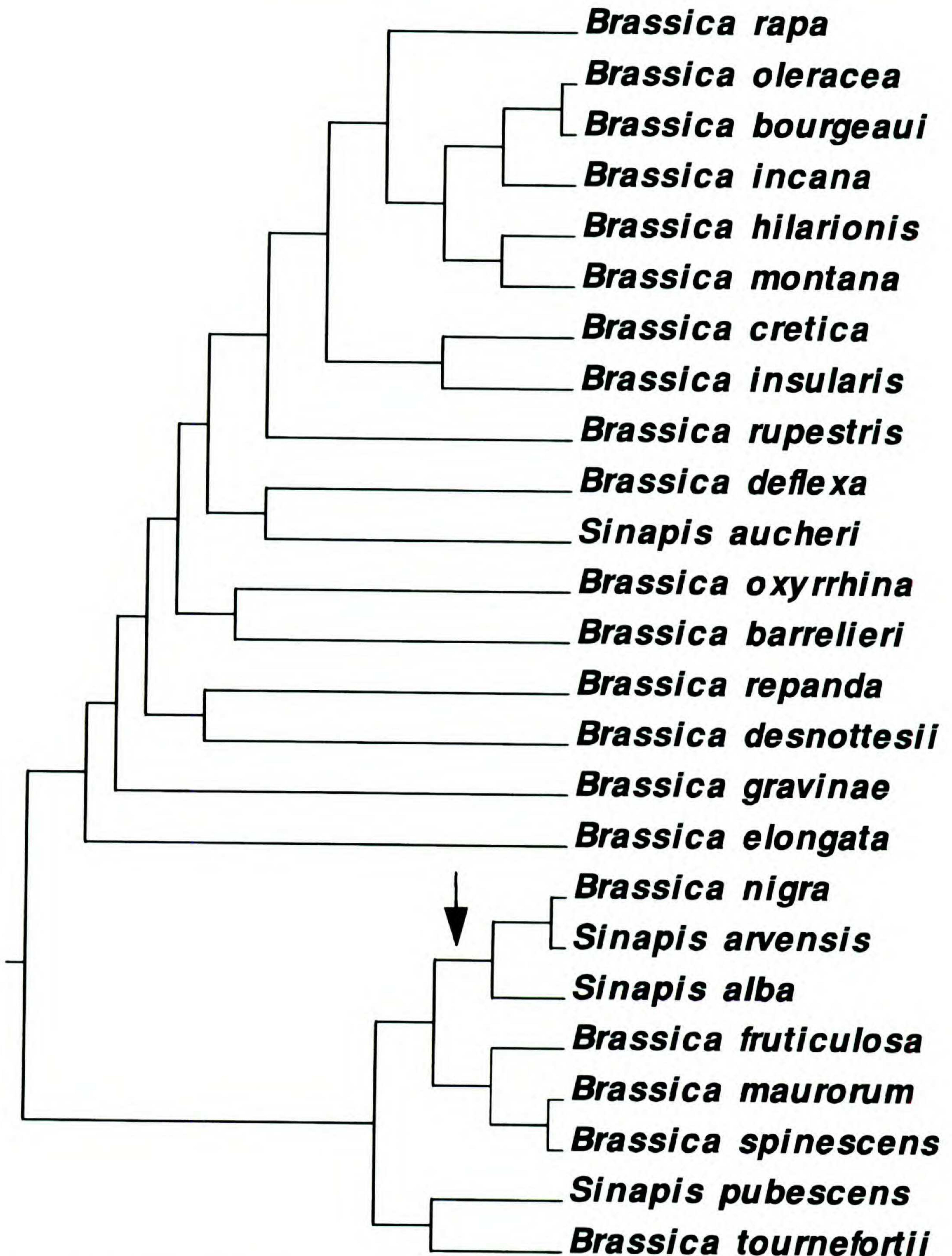
Figure 3. Phylogenetic relationships among members of subtribe Brassicinae (Cruciferae, tribe Brassiceae) based on cpDNA restriction mapping. The tree has been "pruned" from the more complete analysis presented in Warwick and Black (1993), such that only species in *Brassica* and *Sinapis* are shown. The arrow marks the currently known distribution of the inducible PPi-dependent phosphofructokinase as determined by W. Plaxton (Queens University, Ontario, pers. comm.).
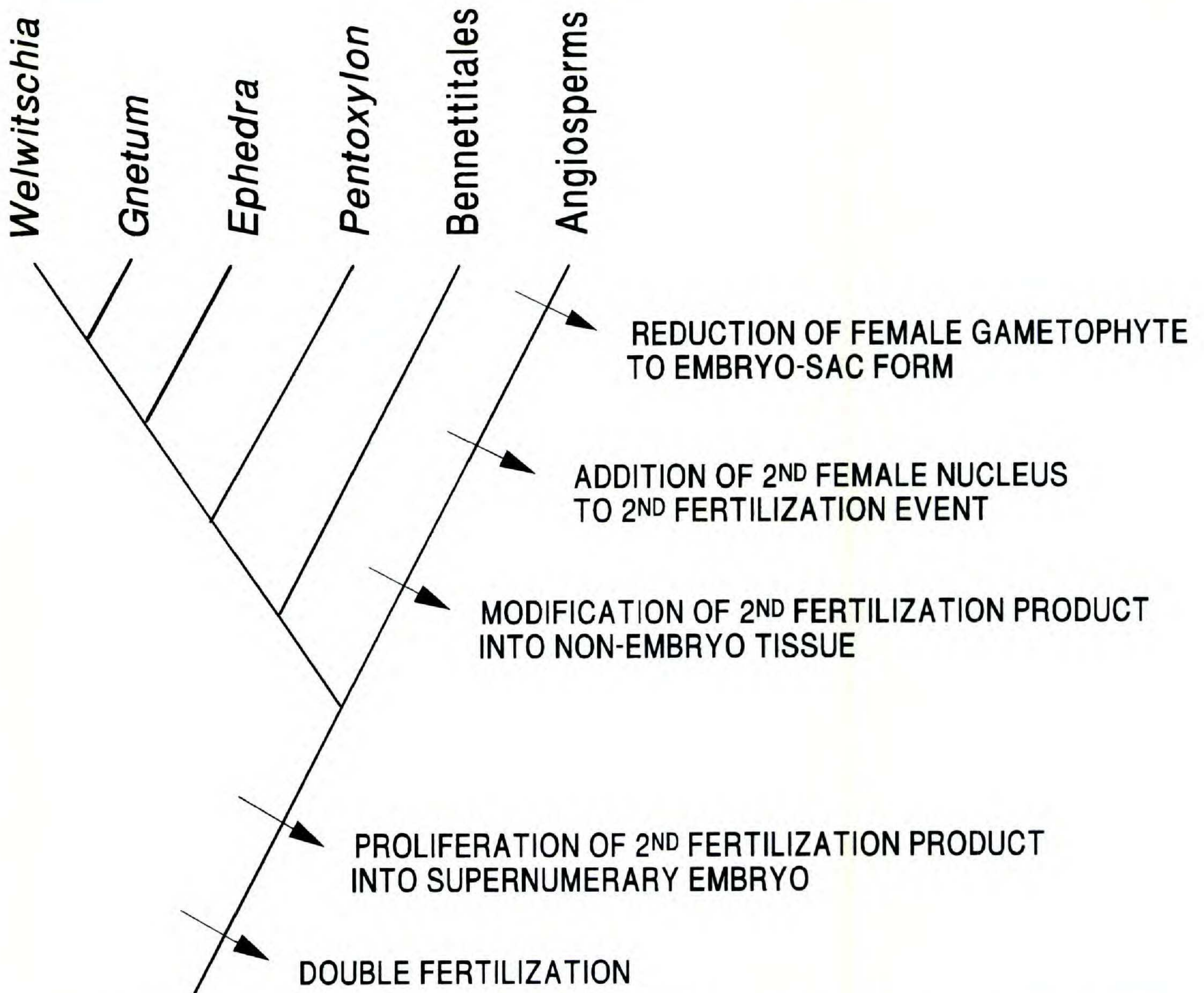
Figure 4. Phylogenetic mapping of double fertilization and the evolution of polyploid endosperm in the Anthophytes, including the Gnetales (*Ephedra, Gnetum, Welwitschia*), the fossil groups *Pentoxylon* and Bennettitales, and the angiosperms. Following observations by Friedman (1990, 1992) it is concluded that double fertilization occurs prior to divergence of the angiosperms, but the evolution of polyploid endosperm occurred after divergence. Redrawn from Friedman (1992).

4). The supernumerary embryo functions to nourish the primary embryo—an act of apparent "altruism" that raises questions about the role of kin selection in the evolution of developmental pathways (Friedman, 1992).

ADAPTIVE RADIATION IN THE HAWAIIAN ISLANDS

Recent collaborative studies between Robert Robichaux (a comparative physiologist and ecologist) and Bruce Baldwin (a comparative molecular systematist) provide an example of how phylogenetic trees can direct the study of physiological adaptation. The Hawaiian silversword alliance includes 28 endemic species that represent three genera (*Argyroxiphium, Dubautia*, and *Wilkesia*). A molecular phylogeny has recently been constructed based on nuclear ribosomal DNA sequences. By mapping the distribution of wet versus dry habitat

preference onto the phylogenetic tree Baldwin and Robichaux (1995) concluded that there have been at least five independent transitions from ancestors occurring in wet habitats to the derived species occurring in dry habitats. Adaptation to dry habitats in this alliance is known to include morphological and physiological traits that influence leaf energy balance (e.g., leaf dimensions, stomatal conductance, and leaf surface properties that regulate solar reflectance) and physiological traits that influence leaf responses to water stress (e.g., cell wall elasticity and its influence on turgor maintenance) (Robichaux et al., 1990). Unfortunately, the molecular phylogeny exhibited its lowest degree of resolution in those lineages with the greatest physiological diversity, limiting the potential for successful reconstruction of physiological diversification. This illustrates one important constraint on

the process of mapping physiology onto phylogenetic trees, namely that one must have a highly resolved phylogeny before gaining insight into physiological divergence.

Using a similar approach, Todd Dawson (a comparative ecophysiologist) has teamed up with Stephen Weller (a comparative systematist), Warren Wagner (a comparative systematist), and Ann Sakai (a population biologist) to study another group of Hawaiian plants, *Schiedea* and *Alsinidendron* (Caryophyllaceae). They have also observed the wet-to-dry transition in this group (Weller & Sakai, 1990; Weller et al., 1990) and are currently investigating the physiological and ecological attributes (e.g., photosynthetic water-use efficiency, hydraulic properties of the water-conducting system) of these taxa which permit them to exploit the drier habitats. Ultimately, the study is aimed at placing these attributes into a phylogenetic context using character mapping procedures and the current phylogenetic tree (Wagner et al., 1995). This will hopefully provide insight into whether those traits that permit certain species to thrive in dry habitats have arisen through response to selection in their current habitats or are historical artifacts of past selection.

EVOLUTION OF C_4 PHOTOSYNTHESIS

One of the earliest uses of phylogeny to discern evolutionary pattern in a functional plant trait involves C_4 photosynthesis. Within five years after the discovery of C_4 photosynthesis researchers had assembled phylogenetic trees showing the distribution of C_4 plants (Evans, 1971; Moore, 1982). From this effort it was clear that C_4 photosynthesis has multiple origins and represents a derived trait, evolving from the ancestral C_3 photosynthetic pathway (the pentose phosphate pathway).

More recently, Hattersley and Watson (1992) constructed an evolutionary hypothesis in the Poaceae that reflects patterns of C_4 evolution and is consistent with well-accepted, higher-level taxonomic affinities. Extensive reticulation of lineages in the Poaceae makes it difficult to reconstruct phylogeny with high levels of confidence (though monophyly has been established for several subfamilies, see Kellogg & Campbell, 1987). Hattersley and Watson (1992) mapped photosynthetic pathways onto their tentative phylogenetic tree, revealing several possible patterns of C_4 evolution. Of particular importance, it appears that multiple origins of C_4 photosynthesis can occur within a single family and reversals of C_4 photosynthesis, back to the ancestral C_3 type, have occurred in several groups. Such patterns suggest that the evolution and rever-

sal of a pathway involving complex developmental and biochemical modifications occurs with relative ease (see also Watson et al., 1985). This supports decisions of optimization during phylogenetic C_4 mapping in which addition and loss of this pathway are treated as equally likely as two additions. It has been suggested that such labile evolutionary patterns must be founded on a genetic architecture with one, or a few, regulatory genes controlling linked sets of C_4-family structural genes (Watson et al., 1985; Monson, 1989a; Hattersley & Watson, 1992; Ehleringer & Monson, 1993). Thus, relatively few mutations, if they occur in the regulatory genes, can have a large influence on the evolutionary expression of photosynthetic pathway type.

Further work on the evolution of C_4 photosynthesis has been conducted in the genus *Flaveria* (Asteraceae). The monograph by Powell (1978) described 21 species for this genus. Physiological characterization of the component species has revealed that only five or six might be classified as "fully expressed" C_3 or C_4 types (Monson, 1989a; Monson & Moore, 1989). Most of the species reflect some intermediate phenotype between the C_3 and C_4 extremes (the so-called C_3-C_4 intermediates). Some workers have described these C_3-C_4 species as intermediate evolutionary stages on the path from the ancestral C_3 type toward the derived C_4 type (Monson et al., 1984; Monson, 1989a; Brown & Hattersley, 1989). Thus, this appears to be a very active group of species in terms of photosynthetic evolution.

Using the morphological descriptions provided in Powell (1978), I have constructed a phylogenetic tree of this genus (Fig. 5). This tree was used to address the question of whether the appearance of C_3-C_4 intermediate photosynthesis always precedes the appearance of fully expressed C_4 photosynthesis. Support for such a pattern would strengthen the supposition that C_3-C_4 intermediate photosynthesis in this group represents the antecedent to the evolution of C_4 photosynthesis. In this reconstruction, C_4 species were classified as those in which the majority of atmospheric CO_2 is assimilated through the C_4 pathway, and the C_3 and C_4 cycles have evolved coordination to the point where leaves exhibit higher water- and nitrogen-use efficiencies— hallmarks of fully expressed C_4 plants. In this case, improvements in photosynthetic water- and nitrogen-use efficiency were assumed if past gas-exchange measurements have revealed reduced intercellular CO_2 concentrations without reduction in photosynthesis rate below the average expressed by all species in the genus. Such traits are presumably a reflection of the C_4 CO_2-concentrating mecha-
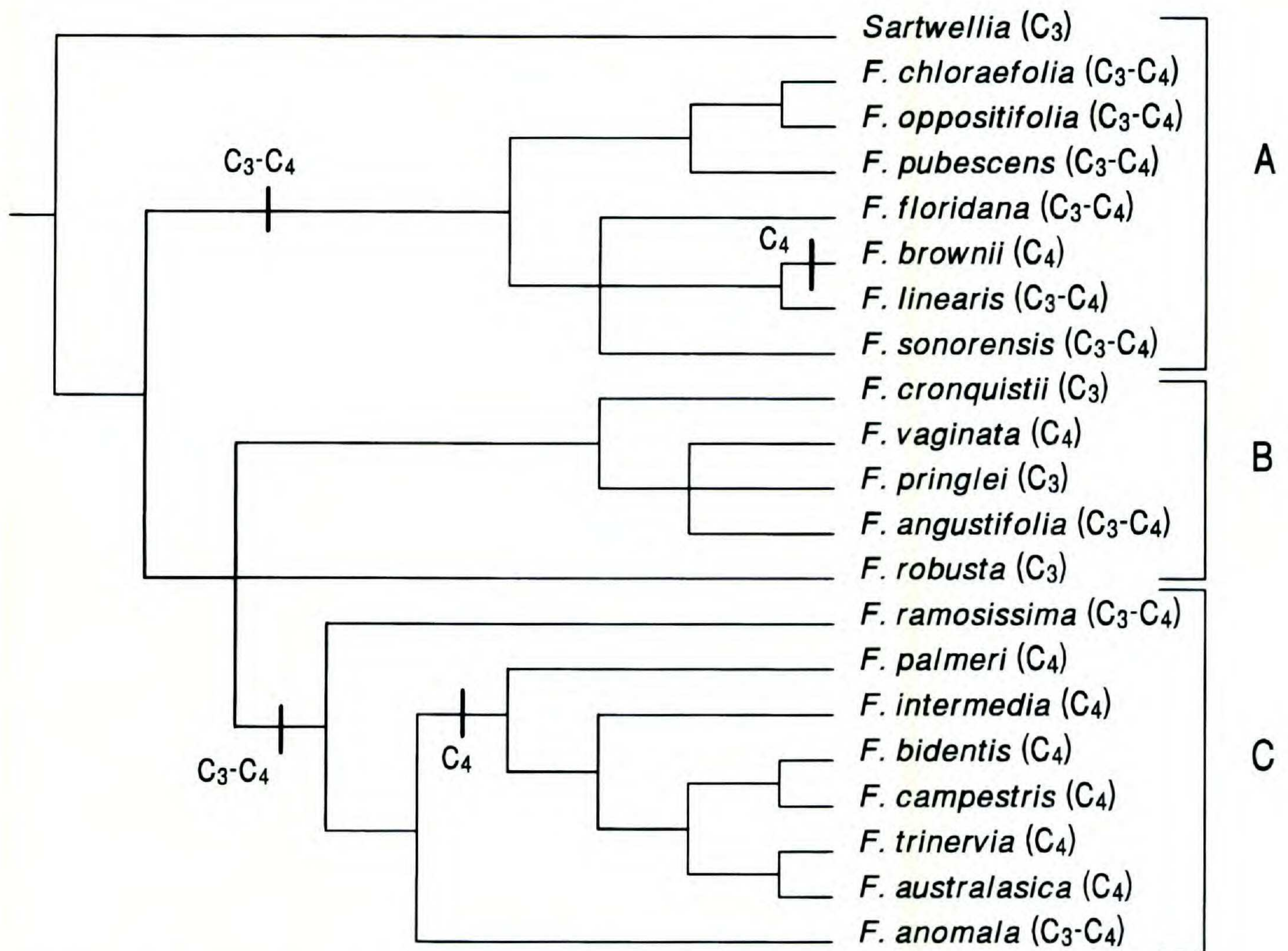
Figure 5. Phylogenetic relationships in the genus *Flaveria* (Flaveriinae–Asteraceae). The tree was constructed using the branch and bound option in PAUP 3.1 (Swofford, 1994). The length of the tree is 33 steps and is characterized by a consistency index (C.I.) = 0.41, a homoplasy index (H.I.) = 0.59, and a retention index (R.I.) = 0.74. All relationships are based on 15 morphological traits that were derived from Powell (1978). Traits included phyllary number, presence or absence of pappus scales, presence or absence of ligules, number of disc florets, presence or absence of discoid heads, length of achenes, density of inflorescence aggregation, presence or absence of self-compatibility, presence or absence of ray florets, overall diameter of the corolla, length of the corolla throat, presence or absence of connate leaves, presence of linear versus ovate leaves, bushy versus erect growth habit, and the presence or absence of leaf spiraling at senescence.

nism. Species with the C_3 pathway are so classified because they exhibit 90% or more of their CO_2 fixation by the C_3 pathway. Species with C_3-C_4 intermediate photosynthesis exhibit considerable CO_2 fixation by the C_4 pathway, but no advantages in terms of elevated water- and nitrogen-use efficiencies. These designations were drawn from past reports of photosynthetic traits in this genus (Ku et al., 1983; Monson et al., 1986, 1987; Moore et al., 1988, 1989; Monson, 1989b; Ku et al., 1991).

The tree exhibits two regions where resolution is such that the sequence of C_3-C_4 and C_4 transitions can be mapped (regions A and C), and one region of inadequate resolution (region B). Using the path of greatest parsimony, the results from regions A and C demonstrate that C_4 photosynthesis has evolved at least twice independently. Additionally, both cases of C_4 evolution were preceded by the

appearance of C_3-C_4 intermediate photosynthesis strengthening the hypothesis that this pathway is an evolutionary precursor to fully expressed C_4 photosynthesis. The tree presented in Figure 5 supports evolutionary lability with respect to the C_4 pathway. Within a relatively small group of species there appear to be cases of independent switches among the C_3, C_3-C_4, and C_4 pathways.

Caution should be used in relying too heavily on this tree to identify details of C_4 evolution. The tree is based on a limited number of characters and only yields moderately robust resolution. A tree based on numerous RFLP molecular markers is currently under construction, and is likely to yield greater resolution in interpreting C_4 evolutionary patterns in this group (P. Soltis and M. Ku, unpublished manuscript; M. Ku, Washington State University, pers. comm.). Nonetheless, the example provided

in Figure 5 demonstrates the utility and guidance provided by phylogenetic perspective in the interpretation of metabolic evolutionary patterns.

CONCLUDING STATEMENT

Since Felsenstein's seminal paper (Felsenstein, 1985), comparative physiologists and developmental biologists have observed the birth of a new experimental approach—the rigorous testing of comparative hypotheses using phylogenetic information. The approach is recent in its origins, and there are only a few examples of its successful application. However, the recent publication of several synthetic treatises of the phylogenetic approach and its relationship to comparative biology testify to the growing interest in this discipline (e.g., Huey, 1987; Harvey & Pagel, 1991; Brooks & McLennan, 1991; Lauder et al., 1995). There is no doubt that comparative biologists will come to rely heavily on phylogenetic perspective in future studies. As so aptly stated in Felsenstein's paper: Phylogenies are fundamental to comparative biology. There is no doing it without taking them into account (Felsenstein, 1985).

Systematics and comparative organismic biology will be reciprocally strengthened through the shared use of phylogenetic analysis. Comparative physiologists and developmental biologists will be able to provide a stronger evolutionary interpretation of functional responses to the environment. Systematists will acquire deeper insight into the evolutionary process, as well as help in resolving points of equivocation on phylogenetic trees through the use of new characters. The most pressing limitation to these activities is the availability of adequately resolved phylogenetic maps. The achievement of a successful synthesis will require the continuation of efforts to construct classification systems that (1) deal with groups holding physiological and developmental interest, and (2) are accurate in their reflection of true phylogeny.

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